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# UNPUBLISHED PRELIMINARY DATA

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## Electron Micrographs of Microspheres from Thermal Proteinoid

Sidney W. Fox and Takeshi Fukushima [1963] 15p refs

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Those interested in the scientific details of the origin of life owe to A. I. Oparin a comprehensive outline of theory dealing with the mechanisms by which many kinds of organic compounds which compose living units might have come into existence (1). Oparin has also emphasized the need for understanding how such biochemical systems might have organized themselves into discrete units. In fact, a dominant aspect of Oparin's own research in this area of inquiry has focussed upon the coacervate droplet (2) as a model of precellular organization. Oparin has pointed out that coacervate droplets are lacking in stability sufficient to constitute an adequate precell model. He has, for example, referred to coacervate droplets as follows: "They lose their structure comparatively easily and go back to the solution state" (3).

An adequate theory of cellular origins therefore calls for either a) a conceptual evolution of the coacervate model to a more stable form, as proposed by Oparin (4), or b) the finding of a more stable experimental model. The latter is at hand in the microspheres which can be produced from thermal proteinoid (5). These microspheres have many unique properties which are not found to be associated simultaneously in any other cell model reported.

The stability of these microspheres is observed in the facts that they retain their original form for weeks or indefinitely, they withstand centrifugation at 3000 revolutions per minute in a clinical centrifuge, and they resist the forces involved in sectioning, as first noted by Miguel, Young, and Munoz with microspheres embedded in blocks of paraffin (6). As Oparin has also pointed out (7) the stability of the organized system correlates with the relative slowness of changes. In the case of the proteinoid microspheres subjected to increased pH, septate cleavage is often observed over a period of between one and two hours (8).

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Stability comparable to that of true cells is more easily conceptualized if the materials from which each is made are comparable. The microspheres are found to be Gram-stainable, and their composition can be varied so that they may be stained either Gram-negative or Gram-positive (9). Furthermore, the solubilities of the Gram-negative and Gram-positive microspheres correlate with the solubilities of Gram-negative and Gram-positive bacteria, respectively (9).

Other properties which have been found and studied in the proteinoid microspheres include: uniformity of microscopic size (10), numerousness (10), responses of swelling and shrinking to salt solutions of appropriate concentration (10), segmentability under pressure (11), phycological modes of association (11), birefringence (11), and a photographed tendency to undergo septate division with change in pH (8).

The stability which permits sectioning permits in turn the preparation of electron micrographs. This paper presents a number of such photographs.

## MATERIAL AND METHODS

The polyamino acid material used for these studies is a 2:2:1 proteinoid prepared by the thermal copolymerization of the eighteen amino acids common to protein (12). For successful copolymerization, the amino acids must contain a sufficient proportion of aspartic acid and glutamic acid, and they must be heated in the absence of solvent water (13).

Microspheres (Fig. 1) are then easily prepared from the polymer of which the detailed preparation has been described (5, 9). Usually, 100 mg. of acid proteinoid is heated with 5.0 ml. of 1.0% sodium chloride solution for 60 seconds and is then allowed to cool. An undissolved residue which is left is larger with polymers of lesser purity. It may also be dissolved progressively by continued contact with hot water. In some cases the hot liquid was filtered into a clean tube, and the latter reheated momentarily to boiling. Upon cooling, in any case, vast numbers of microspheres which are uniform in size are shortly deposited from the hot saturated solution, e.g., $10^8$ microspheres per 10 mg. of polymer (13). These are illustrated in Fig. 1.

Other types of microsphere include one which, upon examination in optical microscope or electron microscope, appears to be hollow. These are typically prepared as follows. One gm. of 2:2:1 proteinoid is treated with 50 ml. of 1.0% sodium choride solution which is brought to and maintained at the boiling point for 5 minutes. The solution is allowed to cool. The microspheres which form then precipitate. McIlwain's buffer (pH 7.0) is added dropwise until the interiors have dissolved as monitored under the microscope. The time for this process varies according to the conditions employed. The final pH tends to be in the range of 5.5-6.5 and the hollowing process may require 30 minutes to many hours.

Microsphere derivatives prepared with lecithin were first hollow

microspheres. These were washed with ethanol, then immersed in an ethanolic solution of lecithin (10% lecithin), and then washed with the supernatant from another preparation of simple microspheres.

For electron microscopy, a suspension of microspheres was stained by standing in a 2.0% solution of osmic acid for one hour. Preparations allowed to stand overnight were not stained to a significantly greater degree.

The methacrylate for embedding was prepared from a 4:1 mixture of butyl methacrylate and methyl methacrylate. After 1 day at $45^{o}$, and 2 days at $65^{o}$, the cured embedded specimen was cut on an LKB Ultratome to yield sections usually 600-800 $A^{o}$ thick. The sections were mounted on copper grids and then examined in a Phillips Model 100 B electron microscope with a Ladd anode.

## RESULTS AND DISCUSSION

Fig. 1 shows simple microspheres approximately 7$\mu$ in diameter. In each of the other figures, the size may be evaluated from the standard short line which is in each instance 1.0$\mu$.

Fig. 2 shows a section of a microsphere which has been treated with buffer solution. Although some bacteria display structure which is considerably more detailed, some bacilli such as Bacillus cereus are comparable in appearance (14). The grainy texture in Fig. 2 is at least superficially comparable to the granular cytoplasm of the true, bacterial cell. Needless to say, the microsphere lacks much that is in the bacterial cell, but the comparability in apparent structure is obvious.

In Fig. 3 are seen five microspheres which have been in a suspension in which the pH has been elevated. In this case various stages of loss of the interior (8) can be observed. This kind of phenomenon is a part of the evidence that the boundary has properties of a true membrane. Both the optical and electronmicrographic results indicate that this boundary has allowed material in the interior to diffuse outward without having itself been displaced.

In Fig. 4 is seen what has been observed under the optical microscope (11) - double layers. These microspheres, also, have been treated with buffer to increase pH. Although double membranes in true cells are generally considered to involve phospholipid, which can alone assemble itself into double layers (15), no phospholipid is present in the material of the microspheres. Lipid quality may, however, be provided by the hydrocarbon sidechains of the neutral amino acid residues within the proteinoid.

Fig. 5 shows also double layers. The larger units have reached a stage from which the internal material has largely dissipated. Also in this figure may be seen a kind of advanced separation of centers described in another paper (8) depicting such phenomena in a time-lapse sequence.

Fig. 6 illustrates a difference in structure sometimes observed when the hollow microspheres are treated with an alcoholic solution containing lecithin. Fig. 7, 8, and 9 are other specimens. In Figs. 7 and 9 are also observed what appear to be hollow centers which are often found in microspheres from proteinoid.

Microspheres stained by uranyl acetate, phosphotungstic acid, phosphomolybdic acid, lead hydroxide, or potassium permanganate did not yield more definitive micrographic results. Embedding in Epon also did not offer advantages.

An attempt to carry microspheres of polystyrene (1.17$\mu$, Dow Chemical Co.) through the total procedure was made. These spherules seemed to take the osmic acid stain poorly or not at all, and also dissolved in the methacrylate used to trap them.

All of the units that have been observed in any one preparation are in the shape and narrow range of size of the microspheres, whereas various "wild" bacteria of course vary over a much greater range of size. The formation of these units are watched under the microscope and they can be made to redissolve by warming. Unlike the microspheres, bacteria do not appear in a few minutes from a clear liquid.

The double layer observed is one of many attributes found in true cells (16). This particular feature had not been sought. No surprise should be engendered, however, by the appearance of unsought cellular attributes in a model which provides other suggestions that it is closely akin to a precellular form of organization.

The properties reported here, elsewhere (5, 6, 8-11), and other unreported properties are found in true cells. Needless to say, other properties and spontaneous type compositions (e.g. RNA) of true contemporary cells are lacking in the model. A mode of thermal polymerization of mononucleotides

similar to that used for amino acids has been partially successful (17, 18) in yielding oligonucleotides. An alternative possibility is that primitive units of organization had many cytological phenomena before they were closely governed by nucleic acids (8).

The self-organizing properties of macromolecular complexes can evidently be found in other kinds of polymers, e.g., polystyrene can form microspheres. To use the polystyrene microspheres as an example, they do not respond to mounting for electron microscopy as do either the proteinoid units or bacterial or other cells. Implicit in the employment of experimental models of the type described is the attempt to approach with increasing closeness the real attributes of the units being modelled. This kind of research is presumed to resemble in mode the spontaneous natural experiments, with one salient difference being that of conscious guidance by human experimenters in the current studies. Either the natural or laboratory model is however subject to improvement by evolutionary or humanly guided experiments respectively.

The microspheres from proteinoid are in one crucial aspect also sharply distinguishable from earlier cell models (2, 19, 20). Many of these models have been made from materials which were in turn prepared from true cells. We can be quite sure that some macromolecules occurring in true cells must indeed have the property of forming the structure of cells. The crucial feature in the present set of observations is that a particular kind of protein-like macromolecule shown to be capable of having arisen easily and spontaneously in the absence of life has the property of organizing itself under simple conditions which would obtain in many locales on many occasions. This property and phenomenon could not have been demonstrated experimentally with polymers derived from cells, but only with those polymers arising in the laboratory from less complex molecules. Such a sequence in the laboratory

enhances the intellectual attraction of a theory of origins in which primitive protein arises first and cells organize themselves subsequently.

A number of thermal polyamino acids exhibit the tendency to form spherical microparticles (13). While it is clear that thermal proteinoid has properties markedly different from those of polymers of markedly different constitution, such as polystyrene, the degree to which the various kinds of polyamino acid possess simultaneously the various properties here recorded and cited is not known. Systematic investigation of this question can be feasibly carried out due to the simplicity of the polymerizations of various mixtures of amino acids [1/].

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[1/] Such laboratory demonstrations call to mind the phrase of Charles Darwin, "---that a proteine compound was chemically formed ready to undergo still more complex changes---" (13).

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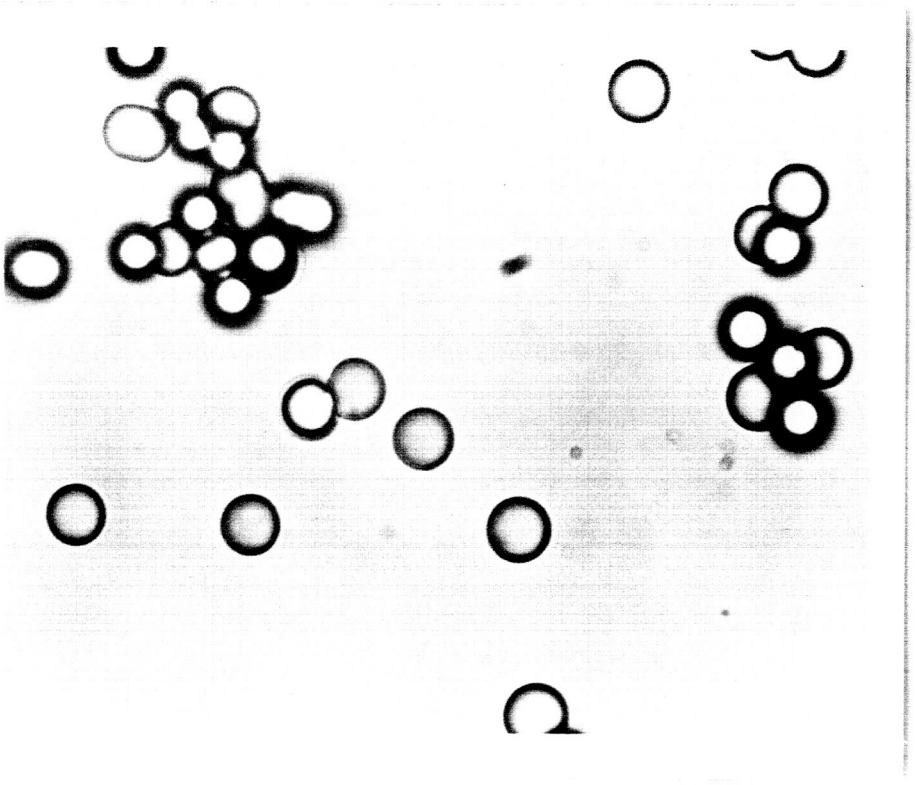
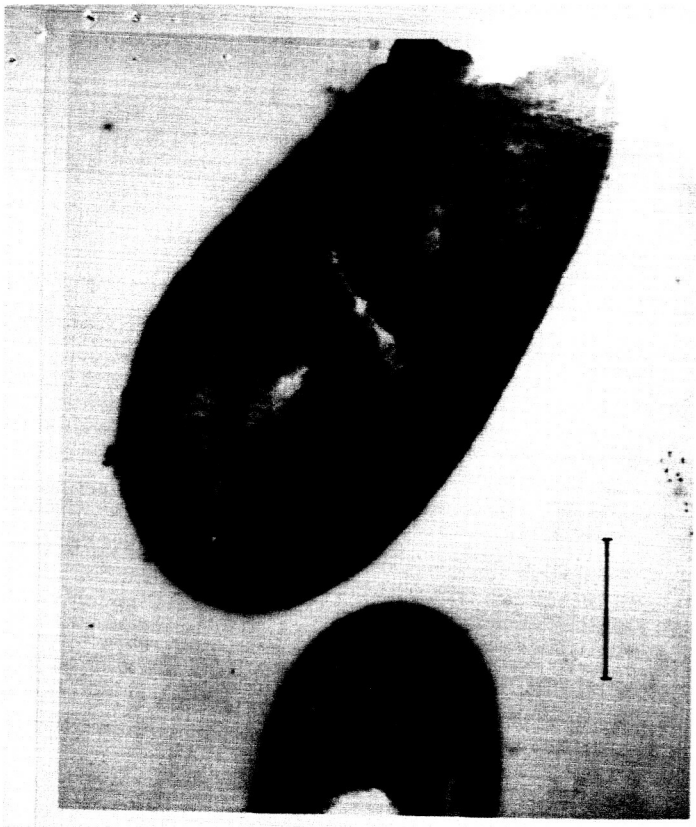


Figure 1



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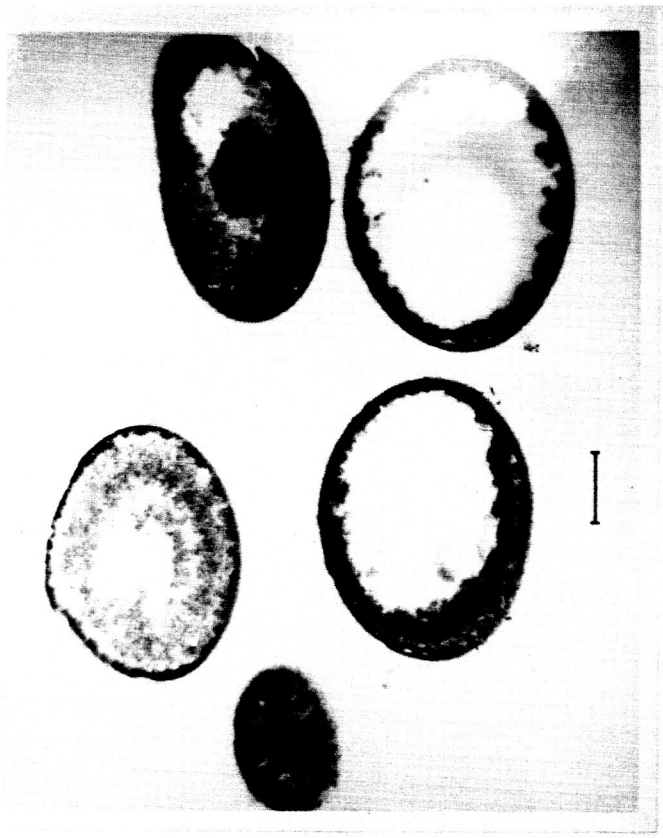

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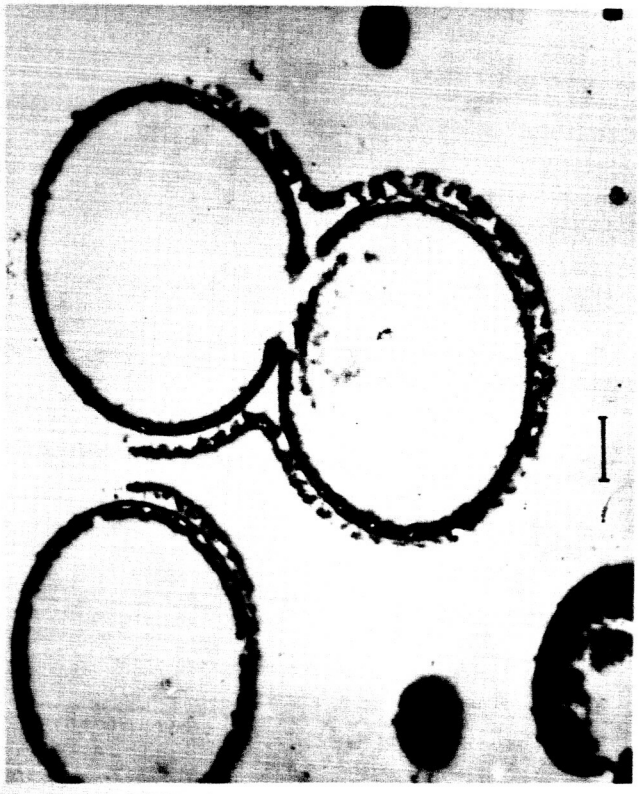

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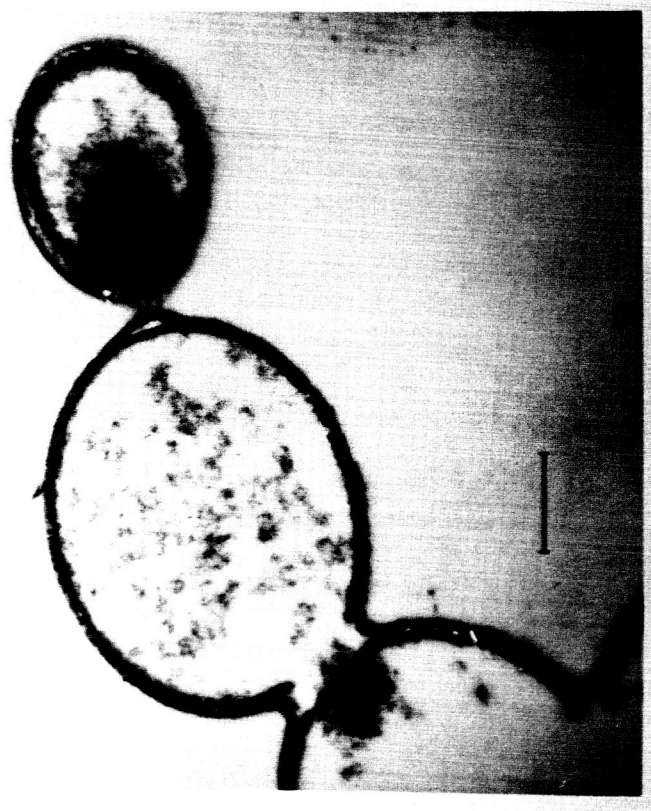
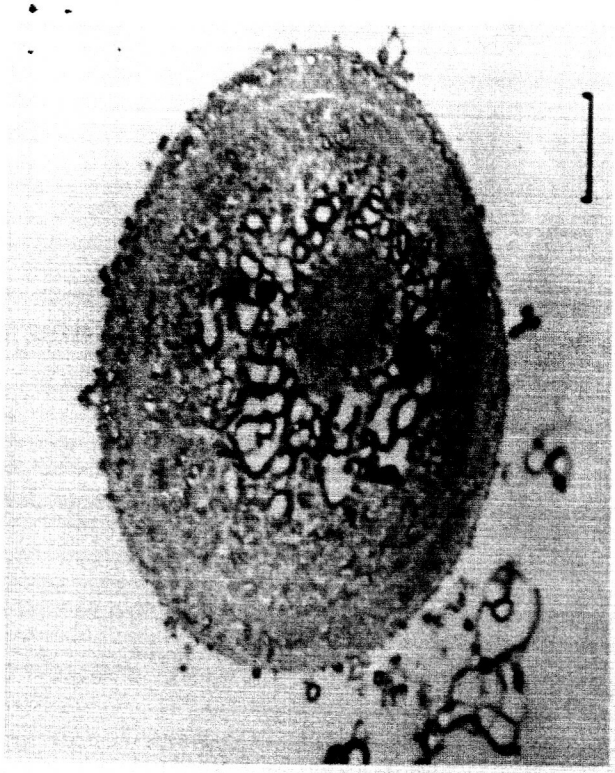


Figure 5



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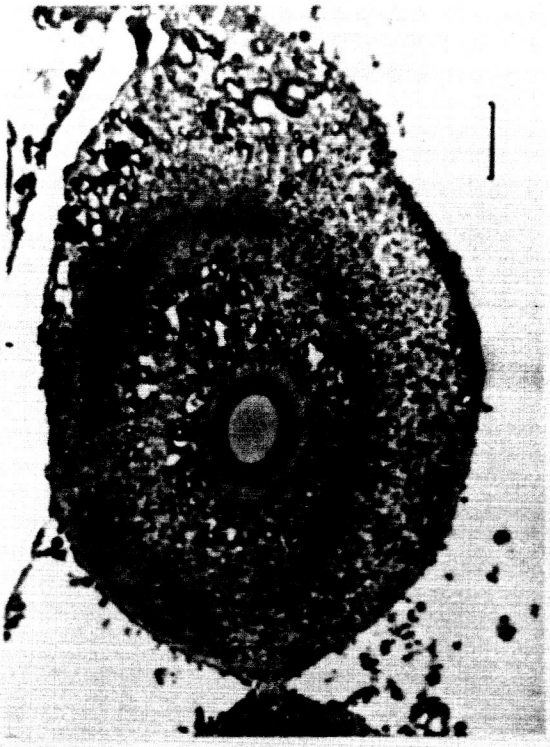

Figure 7

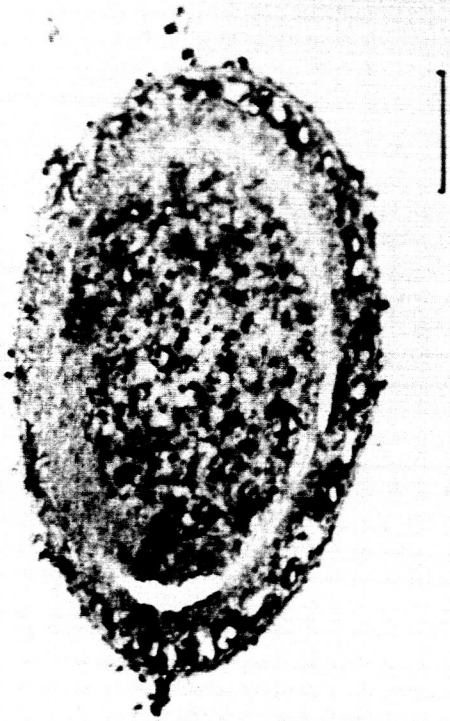

Figure 8

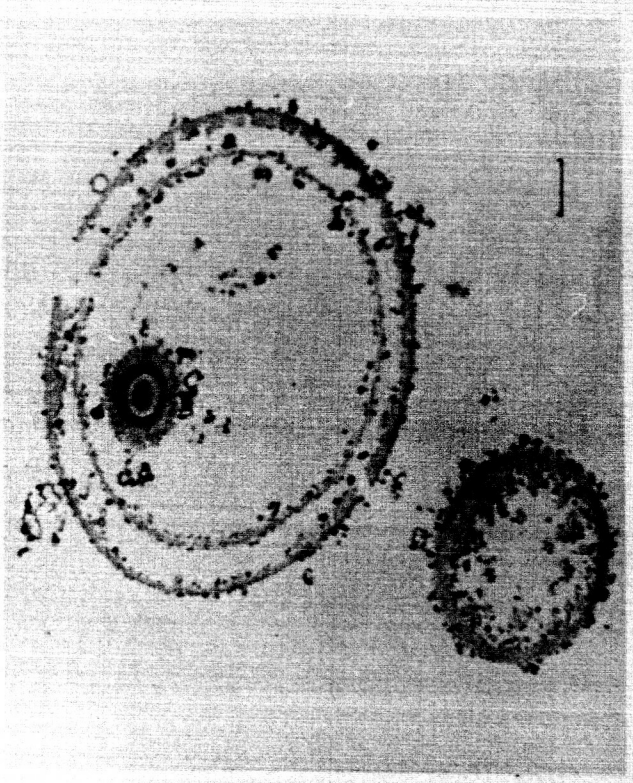

Figure 9